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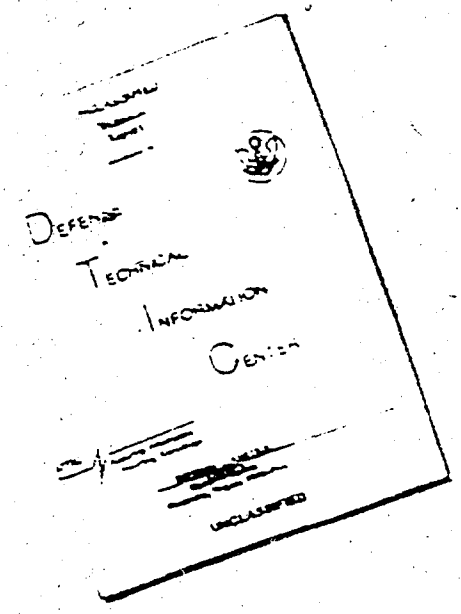

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During the past five years a definitive chemical and pharmacological study has been carried out on the venom of the stingrays, particularly the round stingray Urobatis halleri, common to the Southern California coast. This species is responsible for many hundreds of injuries yearly along California beaches, and its related species are implicated in several thousand injuries per year along North American coastal shores.

The management of stings by these elasmobranchs is unusually successfully instituted by the lifeguard and beach services, who have received training over the years from the Laboratory of Neurological Research. In 1960, 104 cases of stingray injuries were seen at emergency hospital facilities. In 1970, only 27 patients were seen, even though the incidence of reported stings was almost the same. The statistics reveal the success of first-aid measures applied by the beach services.

During the past several years an intensive study has been made of the chemistry and pharmacology of the venom of this fish. Its relative instability has made it difficult, up until this past year, to carry out the necessary isolation characterization and pharmacological studies. However, during the past year the extraction of the venom with 0.05M phosphate buffer containing 10.3M Cleland's reagent has given a product which is stable, and has made it possible to conduct a number of chemical and toxicological studies.

The lethal fraction of the venom is a protein of medium molecular weight, but apparently bound with an enzyme, or some other substance, which makes its separation as a pure substance very difficult. Studies on the purification of the lethal and pain-producing fractions are in progress. Pharmacological studies confirm the earlier findings of Russell and van Harreveld, and indicate that in severe envenomation, secondary shock can occur and can lead to death. The venom has no effect on neuromuscular transmission or on nerve activity, although it produces severe, intense pain.

A series of studies on the *in vitro* neutralization of *Urobatis halleri* venom with antivenins prepared against the bat stingray *Myliobatis californicus* and the round stingray *U. castexi* were carried out. The antivenins were prepared in the conventional manner, that is, by giving successively increasing doses of the crude venom to horses over a 17-week period, maintaining the horses on a maintenance dose for three weeks, then drawing off designated amounts of blood for processing.

The serum was processed by ammonium sulfate precipitation and pepsin digestion, according to WHO requirements.

The venom was prepared by the batch method (Schaeffer *et al.*, Toxicon 9, 69, 1971), reconstituted with 0.05 M phosphate buffer, pH 7.4, in 0.9 percent NaCl at 5° C. The concentration used in all experiments was 10 mg per ml. The extract was centrifuged for 15 minutes at 5,000 rpm and the precipitate discarded. The venom protein concentration was determined and a lethality determination made in mice, using the intravenous route.

One ml of the *M. californicus* antivenin neutralizes the lethal activity of 10 mg of bat stingray venom. One ml of the *U. castexi* antivenin neutralizes the lethal activity of 5 mg of this stingray's venom.

To a number of 1.0 ml samples of *U. halleri* venom, 0.05, 0.20, 0.50 and 1.00 ml of one or the other of the two antivenins were added. Each mixture was agitated then maintained for one hour at 5° C. The mixture was then injected into mice and the effect of the antivenin on survival rate at 24 hours was determined.

The *M. californicus* antivenin provided little protection against the lethal activity of *U. halleri* venom. Only at 1.0 ml of antivenin per ml of venom was there any seemingly significant protection. The *U. castexi* gave some protection at the 0.20 level, the 0.50 level and the 1.00 level. At the

last level the amount of venom required for the LD_{50} had increased threefold. It is apparent that there is some significant cross protection between the antivenins of these two stingray species. Further immunochemical work is in progress on the venoms of four Urobatis species.

Using various methods of extraction, particularly the batch and aspiration methods, as previously described for studies with Scorpaena guttata venom, certain chemical and physiopharmacological properties of the venoms of six species of stingrays were determined.

Employing gel filtration and ion-exchange chromatography it was possible to separate the venom into five biologically active fractions and at least six additional protein fractions, none of which showed a deleterious effect on the cardiovascular survey preparation, the mammalian nerve-muscle preparation, the crayfish nerve-muscle preparation or the frog heart-lung preparation. The principal toxic and lethal fraction appears to be a very unstable protein having a molecular weight of from 300,000 - 800,000. It is unstable in cystine, reduced glutathione and parachloromercuribenzoate, and only slightly less unstable in EDTA. It was more stable in 10^{-3} Cleland's reagent, which was used in some of the separation procedures

The mechanism for the hypotensive crisis produced by lethal doses of the venom and the lethal venom fraction was investigated. Large doses of the venom caused an immediate hypotensive crisis. Although this crisis reflected both direct and indirect actions, the chief effect was on the heart. First, second and third degree blocks were seen in all animals receiving large doses of the venom. Lesser amounts also produced cardiac standstill or damage, as well as marked pooling in the pulmonary circulation (in the cat) and the portal circulation (in the dog). With lesser amounts, cardiac dynamics became a less obvious cause of the hypotensive crisis and the changes in the parameters of

the pulmonary and portal systems appeared to play the more responsive role.

Although the exact mechanism has not yet been determined, it would appear that vasoconstriction on the postcapillary side of the lung is the primary target area. The vasoconstriction leads to a decreased systemic arterial pressure with a subsequent decrease in circulating volume to the right heart. Pulmonary artery pressure is increased while pulmonary flow is decreased. The capillary bed becomes distented and thrombi may be formed, leading to further pulmonary complications. The blood supply to the central nervous system is reduced causing cerebral anemia and changes in the central nervous system, particularly provoking a respiratory deficit. These and other changes in the cardiovascular parameters were investigated.

PLANS FOR FUTURE

Although the contract has been terminated, some further study will be given to the various properties and activities of the venom. A study of the venom-producing cells of the glandular triangle will be made, using the electron microscope.